Answers to questions about the Strep•Tag® II/Strep•Tactin® affinity purification products

What is the principle of Strep•Tag® technology?

The Strep•Tag II purification system is based on the highly selective and easily controlled interaction between the Strep•Tag II peptide and Strep•Tactin®, a specifically engineered streptavidin. The tagged protein binds to immobilized Strep•Tactin during affinity purification. Physiological buffers, like PBS, in combination with a wide range of additives can be used. After a brief wash, purified, active recombinant protein is gently eluted by adding 2.5 mM desthiobiotin to the same buffer. Desthiobiotin is an inexpensive, reversibly binding, and stable analog of biotin—the natural ligand of streptavidin. This competitive elution is the second step conferring specificity, which yields unparalleled, one-step purification. Column regeneration is visualized by a color change on the purification column. For more information, please see page 3.

What is the size of Strep•Tag II and where can it be placed on the fusion protein?

Strep•Tag II is eight amino acids (TrpSerHisProGlnPheGluLys) with a molecular weight of 1 kDa. It can be attached to the N- or the C-terminus, or between two protein domains as a linker. Please see vector information on page 4.

What degree of purity can be expected?

Over 95%. However, impurities resulting from non-specific interactions with the recombinant protein itself could lead to lower purity. To reduce contaminants covalently linked to the recombinant protein by disulfide bonds, add reducing agents to all buffers for cell lysis and chromatography. For non-covalently linked contaminants, increase the ionic strength in all buffers for cell lysis and chromatography by adding NaCl or mild detergents (e.g., TRITON® X-100, TWEEN® 20, CHAPS.)

Does Strep•Tag II bind avidin?

No. Therefore, avidin can be used to block naturally occurring biotin in cell lysates and from culture medium.

For proteins expressed in the cytoplasm, is the presence of biotinylated proteins in the host organism a problem?

No. Generally, the amount of biotinylated proteins in the cytoplasm is very low and does not lead to significant inactivation of the column. In an *E. coli* extract derived from a 1 L culture with $OD_{550} = 1$, the total biotin content is only around 1 nmol; column capacity is 350 nmol/ml. Even the biotinylated *E. coli* biotin carboxyl carrier protein (BCCP) has a relatively low intracellular concentration and usually does not interfere with purification. However, to avoid binding biotin irreversibly to Strep•Tactin resin, add avidin to the cell lysate before chromatography (20 µg/L for an *E. coli* culture at $OD_{600}=1$).

For secreted proteins, is the presence of free biotin in the medium a problem (eukaryotic expression)?

Yes, it can be. The amount of biotin present depends on the cell line and the medium. Some media for insect cells or mammalian cells contain up to 800 nmol biotin per liter that has to be removed or blocked before applying the lysate to Strep•Tactin columns. For mammalian cell culture only DMEM and Leibovitz's L-15 media are free of biotin. For insect cell culture, only Schneider's medium is free of biotin. Ingredients of proprietary formulations for serum free growth are usually not disclosed, but information on biotin content can be obtained, and should be requested, from the manufacturer.

When the protein elutes from the column, is it complexed with desthiobiotin?

No. Desthiobiotin does not complex or interfere with the protein or general protein assays, and can be removed by gel filtration or dialysis.

Is it possible to detect protein-protein interactions using Strep•Tag® II technology?

Yes. The One•STrEP™ Protein Interaction Kit (Cat. No. 71624-3) was specifically designed to isolate intact protein complexes.

Is there a convenient method for parallel purification of different Strep•Tag II proteins?

Yes. The Strep•Tactin® HT96™ Purification Kit (Cat. No. 71605) was designed for automated, high-throughput purification of Strep•Tag II proteins (up to 100 µg Strep•Tag II protein per well). The plates are pre-loaded with Strep•Tactin affinity resin and simply have to be rehydrated and equilibrated before use. The plate can be used with standard vacuum manifolds for manual sample processing or is compatible with robotic sample processing systems.

Can the Strep•Tag II be removed?

Yes. Please see vector information on page 4 to find vectors encoding enterokinase (Ek) or HRV 3C cleavage sites. However, due to the small size and chemically inert nature of Strep•Tag II, it generally does not interfere with the folding or bioactivity of the recombinant protein and does not need to be removed.

Can detergents or other buffer systems be used?

Yes. As long as the pH remains above pH 7.0, high salt, reducing reagents, chelating reagents, and detergents can be used with Strep•Tactin. The resin is also compatible with BugBuster® Protein Extraction Reagent (Cat. No. 70584) and high protein yields have been achieved using BugBuster Master Mix (Cat. No. 71456) to prepare cell lysates. The following table lists reagents that have been successfully tested.

Reagent	Concentration
Reduction Agents	
DTT	50 mM
2-mercaptoethanol	50 mM
Non-ionic Detergents	
C_8E_4; Octyltetraoxyethylene	0.88 %
$C_{10}E_5$; Decylpentaoxyethylene	0.12 %
$C_{12}E_8$; Octaethyleneglycol Mono-*n*-dodecyl Ether	0.005 %
$C_{12}E_9$; Dodecyl nonaoxyethylene (Thesit)	0.023 %
Decyl-β-D-maltoside	0.35 %
N-dodecyl-β-D-maltoside	0.007 %
N-nonyl-β-D-glucopyranoside	0.2 %
N-octyl-β-D-glucopyranoside	2.34 %
Triton X-100	2 %
Tween 20	2 %
Ionic Detergents	
N-lauryl-sarcosine	2 %
8-HESO; N-octyl-2-hydroxy-ethylsulfoxide	1 %
SDS; Sodium-N-dodecyl sulfate	0.1 %
Zwitterionic Detergents	
CHAPS	0.1 %
DDAO; N-decyl-N,N-dimethylamine-N-oxide	0.034 %
LDAO; N-dodecyl-N,N-dimethylamine-N-oxide	0.13 %
Others	
Ammonium sulfate $(NH_4)_2SO_4$	2 M
$CaCl_2$	1 M
EDTA	50 mM
Ethanol	10 %
Guanidine	1 M
Glycerol	25 %
Imidazole	250 mM
$MgCl_2$	1 M
NaCl	5 M
Urea	1 M

Note: These reagents have been successfully tested for purification of GAPDH-Strep•Tag II with concentrations up to those mentioned. Since binding depends on the sterical accessibility of Strep•Tag II in the context of the particular protein, concentrations may deviate from the given value for different proteins.

How, and how many times, can Strep•Tactin be regenerated?

The matrix is regenerated with an azo dye, hydroxyl-azophenyl-benzoic acid (HABA), which, when applied in excess, displaces desthiobiotin. The dye is yellow in solution and shifts to red when bound by Strep•Tactin, allowing visual control of the regeneration process and the functional status of the column. As long as a color gradient between the top and the bottom of the column is visible, it is not fully regenerated. The resin can be regenerated 3 to 5 times.